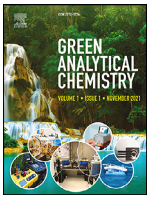
Green Analytical Chemistry 5 (2023) 100056

Contents lists available at ScienceDirect

Green Analytical Chemistry

journal homepage: www.elsevier.com/locate/greeac



How to evaluate methods used in chemical laboratories in terms of the total chemical risk? – a ChlorTox Scale

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ARTICLE INFO

Keywords:
Chloroform
ChlorTox Scale
Green Chemistry
Green Analytical Chemistry
Risk assessment
Sample preparation
White Analytical Chemistry

ABSTRACT

Reliable assessment of the health and environmental risk associated with the use of chemicals is critical to objectively evaluate the greenness and whiteness of an analytical method. An objective and useful way of risk assessment should take into account all the relevant properties of used substances, their actual amounts needed for application of the method, and quantify the assessment result using a standardized unit. This article presents a relatively simple approach to meet the above requirements, which we call the Chloroform-oriented Toxicity Estimation Scale (ChlorTox Scale). The risk posed by a given substance is expressed by the ChlorTox value, and the sum of the values obtained for all reagents is the overall risk posed by the method (Total ChlorTox). The ChlorTox value is calculated by comparing the hazards for the substance being assessed to the standard – chloroform, using the approach selected by the user as optimal, taking into account the amount of a pure substance needed for single analysis. The ChlorTox unit is the equivalent mass of chloroform which reflects the estimated degree of predicted risk. This tool can be used for risk assessment in laboratories of various profiles.

1. Introduction

The main challenges facing analytical chemistry today as a science and field of human activity include the need to follow and implement the ideas of Green Analytical Chemistry (GAC) and White Analytical Chemistry (WAC). GAC tends to reduce the impact of analytical methods on the natural environment and their users [1–3], while WAC tends to reconcile the greenness with unbiased functionality (analytical performance and practicality) [4]. Taking into account the huge number of diverse laboratories performing chemical analyzes all over the world, the actual scale of this challenge seems very large [5].

There are several parameters determining the greenness of methods used in the chemical laboratory [6]. One of them is the energy demand related to powering research equipment and laboratory infrastructure, which results in carbon dioxide emission. Its importance has been discussed in a paper recently published in Green Analytical Chemistry [7]. The present article focuses on another criterion, which undeniably, plays the fundamental role in GAC. It can be called "chemical risk", and its

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Abbreviations: CSDF-ME/GC–MS, continuous sample drop flow microextraction with gas chromatography coupled with mass spectrometry; DBS-MAE/CE-MS, dry blood spot and microwave assisted extraction with capillary electrophoresis coupled with mass spectrometry; DBS-MAE/UHPLC-MS, dry blood spot and microwave assisted extraction with ultrahigh performance liquid chromatography coupled with mass spectrometry; DI-SPME/GC-MS, direct immersion solid phase microextraction with gas chromatography coupled with mass spectrometry; DI-SPME/UHPLC-MS, direct immersion solid phase microextraction with ultrahigh performance liquid chromatography coupled with mass spectrometry; GAC, green analytical chemistry; GC, gas chromatography; HPLC, high performance liquid chromatography; HSDB, hazardous substances data bank; HS-SPME/GC-MS, head space solid phase microextraction with gas chromatography coupled with mass spectrometry; LLE/HPLC, liquid-liquid extraction with high performance liquid chromatography; MAE/HPLC, microwave assisted extraction with high performance liquid chromatography; MAE/UHPLC-MS, microwave assisted extraction with ultrahigh performance liquid chromatography coupled with mass spectrometry; MEPS/UHPLC, microextraction on packed sorbent with ultrahigh performance liquid chromatography; MS, mass spectrometry; MSPE/GC–MS, magnetic solid phase extraction with gas chromatography coupled with mass spectrometry; RSE/GC–MS, rotating sorptive extraction with gas chromatography coupled with mass spectrometry; PE-DLLME/GC–MS, solid phase extraction and dispersive liquid-liquid microextraction with gas chromatography coupled with mass spectrometry; SS-LPME/GC–MS, switchable solvent-liquid phase microextraction with gas chromatography coupled with mass spectrometry; WAC, white analytical chemistry WHN, weighted hazards number.

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https://doi.org/10.1016/j.greeac.2023.100056
Received 28 February 2023; Received in revised form 11 April 2023; Accepted 12 April 2023

meaning is indeed quite broad. It consists of a number of factors, such as the risk of transport and storage of the substance, the risk of toxic effects on the method's user during unavoidable contact, the risk of unplanned contact due to an unforeseen emergency situation, the risk of release of the substance into the environment during the routine use of the method or emergency situation causing contamination of biosphere, as well as the risk associated with the process of storage and disposal of the generated waste. All these risks are determined by the severity of specific hazards posed by chemicals and the exposure dependent on their quantity.

It is obvious that the use of dangerous substances in chemistry cannot be completely eliminated in a short term, nevertheless, effort can be made to increase the awareness of hazards related to their utilization, and to search for less toxic alternatives. The observed rapid growth of interest in such efforts is very encouraging [5]. The toxicity of chemicals used and their amounts are quite often discussed in the present literature, especially when publishing new analytical methods. They are considered by the popular greenness and whiteness metric tools [8–16], however, their assessment is simplified and based on some important generalizations. Undoubtedly, only an approach taking into account the detailed characteristics of hazards for the assessed substance, as well as its accurately estimated quantity, can allow for an objective assessment of risk and method evaluation.

Unfortunately, there is no ideal way of quantifying and expressing the overall hazard posed by various chemicals that would allow for the reliable and simple risk assessment. The fact is that the more accurate and objective we try to be, the more complicated the model will become and the more difficult will be its smooth implementation. Choosing the right approach can therefore be a non-obvious and disputable matter. On the other hand, it seems advisable to unify the assessment procedure and develop one standard protocol of method evaluation that would allow comparisons of methods to be made more transparently and on a larger scale. In addition, it seems advisable to introduce a unified unit of chemical risk assessment that would facilitate its visualization and interpretation. Such common unit would also allow to establish the requirements that a given type of method must meet in order to be classified as "formally" green. In the future, such parameter could be included in the pool of mandatory validation criteria.

2. ChlorTox Scale

In this article we would like to present a novel indicator of chemical risk, which partially eliminates the aforementioned limitations. We call it Chloroform-oriented Toxicity Estimation Scale (ChlorTox Scale). The key assumption is to refer to the reference substance which we selected for this purpose – chloroform. It is a well-known and thoroughly tested chemical substance in terms of toxicity and safety, which poses many potential hazards of various nature to the user and the environment, therefore it requires the use of adequate personal protective equipment, hazard prevention and detailed characterization in readily available risk assessment sheets. In addition, chloroform is offered by many different manufacturers, and thus there are many different sources of data on its properties in the form of safety data sheets, which are regularly updated.

The basis of our approach is to estimate the overall chemical hazard for the substance-of-interest (CH_{sub}), and refer it to the overall chemical hazard posed by the standard – chloroform (CH_{CHCl3}). Secondly, it is required to reliably calculate and consider the mass of the substance-of-interest needed for a single analysis/measurement (m_{sub}):

$$\text{ChlorTox} = \frac{CH_{sub}}{CH_{CHCl3}} \cdot m_{sub} \tag{1}$$

Where the ChlorTox value, expressed in the mass of chloroform [g], reflects a degree of chemical risk associated with the substance-of-interest, taking into account its properties (hazards) and the amount used.

CH_{sub}/CH_{CHCl3} represents a relative hazard of using the assessed substance in relation to chloroform, assuming the same mass-to-volume concentration of both chemicals. The ChlorTox values characterizing different substances can be added together to express the total chemical risk predicted for the whole method (Total ChlorTox).

The ChlorTox value has a purely theoretical meaning, it is not directly reflected in reality, but it indicates the general scale of potential risk. For example, a method with a Total ChlorTox value of 1 g poses a risk which is analogous to a method using 1 g of pure chloroform per one analysis as the only dangerous chemical reagent. Similar approaches to the theoretical expression of risks for the selected group of contaminants, based on toxic equivalency factors, are already known, and used in environmental toxicology [17,18].

When estimating the mass of the reagents used, the conversion to the pure (most concentrated) form should always be performed. For majority, pure form will mean 100% content of a given chemical compound. In some cases this will mean a lower content, for example for ammonium hydroxide (ammonia water) the highest achievable concentration is about 35%, and for hydrochloric acid 36%.

It crucial to realize that performing of even single analysis (measurement) may require preparation of instruments, calibration of the method, and rinsing of instruments after the measurements are completed. The appropriate amounts of reagents required for these steps should be taken into account to avoid underestimation of risk for a whole method. On the other hand, assuming a need for preparation and calibration of the method only for one measurement may lead to the opposite effect – overestimation of risk. Each methodology has its own specifics, thus the number of measurements that can be performed in a consecutive series after preparation and calibration varies significantly. Therefore, we propose to use the following formula for estimating the mass of the substance needed for single analysis (m_{sub}):

$$m_{sub} = \frac{m_N + m'}{N} \tag{2}$$

Where m_N is the mass of the substance consumed directly when performing a series of N consecutive analyses, and m' is the mass of the substance consumed when performing additional mandatory steps, like calibration and rinsing (not direct sample processing). N should reflect the longest possible series without a need for performing additional steps, providing expected (unbiased) quality of results. The N value should characterize the particular methodology and be the same for all reagents used at the same stage of analytical procedure. Extractions and other sample preparation methods may have own N value, depending on their characteristics. In the case of substances used only in direct sample processing (its preparation or analysis), m' equals zero. In the case of substances used only at additional steps, e.g. solvents used only to rinse the equipment, m_N equals zero.

In the case of liquids and gasses, it may be necessary to convert their measured volume to mass using the density value. In the case of liquids, densities are easily available as it is the basic physical parameter. Exceptionally, if the relevant data are not available, we propose to take the density of another substance very similar in terms of properties, or ultimately, the density of water, i.e. 1 g/mL.

In the case of gasses, accurate measuring of mass may be technically difficult. One should also remember about different specificity of the hazards posed by them. They are often associated only with their high compression in cylinders or deep refrigeration, not chemical reactivity. These are physical hazards. Because the ChlorTox Scale is aimed at assessing typically chemical risk, the hazards associated with the explosion of non-inflammable chemicals or cryogenic burns and injury caused by extremely low temperature (hazards denoted in safety data sheets as H280, H281, H284) should not be included in the assessment. Nevertheless, gasses must be included if they exhibit toxic, flammable, or oxidizing properties resulting from their chemical nature. Then, performing the appropriate approximation of their mass consumed for analysis is advised.

In the case of newly developed and commonly unknown chemical reagents, access to key data defining their properties may be impossible. In such a situation, it is recommended to assess the hazards in a simplified way, for example by referring to other substances with a similar chemical structure, better described in terms of properties.

To facilitate the use of the ChlorTox Scale, the analyte standards used in the method calibration process do not need to be included in the assessment process, if their influence on the Total ChlorTox value is negligible. The same applies to the solvents used to prepare standard solutions. If their amounts are negligible, omitting them from the assessment will not significantly affect the final outcome. We propose to make the assumption that standards and solvents for their preparation can be omitted if the resulting ChlorTox value does not exceed 0.1 g. It should be taken into account how many calibrations and sample measurement can be performed with the prepared standard solutions (according to Eq.2, this factor directly affects the resulting ChlorTox value). According to our general predictions, this condition should be met in most cases and standard solutions could usually be excluded from the assessment. Moreover, in most cases, different methods for the same purpose will require the preparation of a comparable set of standards, therefore this factor will not be relevant in comparing methods and identifying significant differences between them.

Water as a completely safe substance is by definition not included in the assessment. If the preparation of water or aqueous solutions with appropriate characteristics requires the use of chemicals, they should obviously be included as separate reagents. Other substances with a minor contribution to the ChlorTox value should not be excluded from the analysis, as this information may be important in comparing methods and identifying reagents worth and not worth replacing for risk minimization.

Importantly, Eq.1 does not indicate the model of estimating the values of CH_{sub} and CH_{CHCl3}, in other words, it gives the freedom of choosing the optimal hazard estimation approach. Depending on the situation, it may differ in type of input data, mathematical structure, and the degree of complexity. Nevertheless, it is essential to use the same model for the chemical under consideration and the chloroform used as a standard for assessing that chemical. The lack of imposing rigid guidelines enables finding the best compromise between meticulousness and user-friendliness in varied situations, furthermore, may stimulate the evolution of hazard estimation models dedicated to ChlorTox Scale in the future.

3. Hazard assessment models

3.1. Weighted hazards number (WHN)

To facilitate rapid evaluation of the method using the ChlorTox Scale, we have developed a simple model for quantifying general chemical hazard, called the Weighted Hazards Number (WHN). It consists in searching for relevant information on the hazards posed by given chemical reagents in publicly available safety data sheets, presented in the commonly used Globally Harmonized System of Classification and Labeling of Chemicals (GHS) format. The GHS format was developed and established by the United Nations at the beginning of the 21st century. In accordance with the general directive, hazards are identified on the basis of the commonly adopted guidelines and marked with the appropriate word and picture code (pictograms) in the safety data sheets, using letter "H" followed by a number to point individual hazard. This system covers hazards associated with storage and transport, direct health hazards (poisoning, chemical burns, irritation, carcinogenicity) and environmental hazards (impact on model species of microorganisms, plants, and animals). In addition, there are categories denoting the degree of hazard, the number of which, depending on the type of hazard, ranges from 1 to 4. Category 1 means the highest degree of hazard (the greatest potential danger), while 4 the least. This information is always presented in the Section 2 (Hazards identification).

For example, for chloroform offered by Sigma-Aldrich one can find the following information [19]: Acute toxicity, Oral (Category 4), H302. Acute toxicity, Inhalation (Category 3), H331. Skin irritation (Category 2), H315. Eye irritation (Category 2A), H319. Carcinogenicity (Category 2), H351. Reproductive toxicity (Category 2), H361. Specific target organ toxicity - single exposure (Category 3), Central nervous system, H336. Specific target organ toxicity - repeated exposure, Oral (Category 1), Liver, Kidney, H372. Short-term (acute) aquatic hazard (Category 3), H402. Thus, nine hazards in total have been identified, of which one is category 1, four are category 2, three are category 3 and one is category 4.

In the WHN approach, the overall hazard of the substance-of-interest (CH_{sub}) is determined as the sum of the hazards identified in the Section 2 of the safety data sheets (GHS format), with weights reflecting the degree of potential danger (hazard category): 1 for category 1, 0.75 for category 2, 0.5 for category 3 and 0.25 for category 4:

$$CH_{sub} = 1 \cdot N_{cat1} + 0.75 \cdot N_{cat2} + 0.5 \cdot N_{cat3} + 0.25 \cdot N_{cat4} \quad (3)$$

Where N_{cat} is the number of hazards of a given category.

For chloroform, according to the data provided in safety data sheet supplied by Sigma-Aldrich [19], the CH_{sub} value should be calculated as:

$$CH_{sub} = 1 \cdot 1 + 0.75 \cdot 4 + 0.5 \cdot 3 + 0.25 \cdot 1 = 1 + 3 + 1.5 + 0.25 = 5.75 \quad (4)$$

The procedure for estimating the CH_{sub} value according to the WHN model is also illustrated in Fig. 1.

Because in the ChlorTox Scale chloroform plays the role of universal standard for estimating relative hazard of other chemicals, this value should be used in Eq.1 as CH_{CHCl3}. In consequence, the relative hazard characterizing the substance-of-interest would be defined as:

$$\frac{CH_{sub}}{CH_{CHCl3}} = \frac{CH_{sub}}{5.75} \quad (5)$$

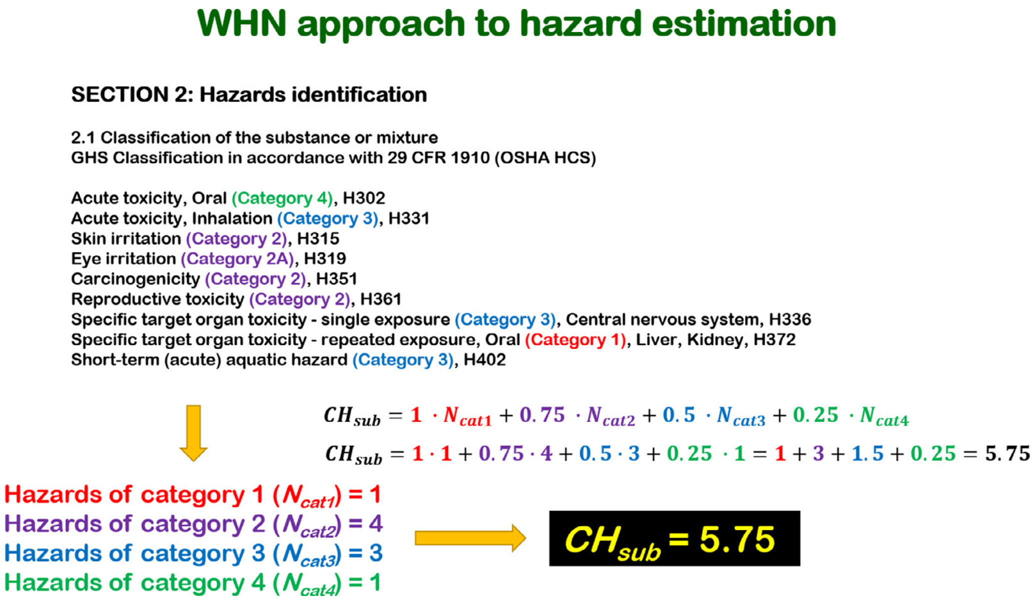
Thus, a substance with a CH_{sub} value less than 5.75 will be in theory less hazardous than chloroform, and greater than 5.75, more hazardous. A substance half as hazardous as chloroform should have this value close to 2.9, etc. It is also worth noting that the CH_{CHCl3} value may be different if choosing a different supplier of safety data sheets (different than Sigma-Aldrich). It should be taken into account that the GHS system is still relatively new and allows some flexibility in interpreting the formal guidelines related to hazard identification. Therefore, the potential discrepancies observed between various manufacturers can be expected.

To ensure reliable assessment with the WHN model, our recommendation is to select one preferred safety data supplier for the whole assessment process. To keep transparency, its name should always be given in the assessment description. The selected supplier should allow for hazard assessment of as many reagents as possible. It should also provide data for chloroform. The resulting CH_{CHCl3} value should be used consistently for assessing relative hazards of all reagents.

Noticeably, different manufacturers often have the same supplier of safety data, and this helps to maintain data consistency (check the information presented in Section 1 of GHS safety data sheet about data supplier). Moreover, it is always recommended to refer to the most updated version of sheet available. One should also verify each time if the data refer to the right form of substance (the form to which the value of m_{sub} used in Eq.1 refers). For reagents for which the selected supplier does not provide any up-to-date sheet prepared in GHS format, we recommend using most updated safety data sheet provided by other suppliers. In order to find all required information very quickly, the search engine available on the chemicalsafety.com [20] or other websites can be used.

3.2. CHEMS-1

The CHEMS-1 model was originally used to assess chemical risk in industry [21], afterwards, in 2015 it was used by Tobiszewski and



WHN approach to hazard estimation

SECTION 2: Hazards identification

2.1 Classification of the substance or mixture
GHS Classification in accordance with 29 CFR 1910 (OSHA HCS)

Acute toxicity, Oral (Category 4), H302
Acute toxicity, Inhalation (Category 3), H331
Skin irritation (Category 2), H315
Eye irritation (Category 2A), H319
Carcinogenicity (Category 2), H351
Reproductive toxicity (Category 2), H361
Specific target organ toxicity - single exposure (Category 3), Central nervous system, H336
Specific target organ toxicity - repeated exposure, Oral (Category 1), Liver, Kidney, H372
Short-term (acute) aquatic hazard (Category 3), H402

$$CH_{sub} = 1 \cdot N_{cat1} + 0.75 \cdot N_{cat2} + 0.5 \cdot N_{cat3} + 0.25 \cdot N_{cat4}$$

$$CH_{sub} = 1 \cdot 1 + 0.75 \cdot 4 + 0.5 \cdot 3 + 0.25 \cdot 1 = 1 + 3 + 1.5 + 0.25 = 5.75$$

Hazards of category 1 (N_{cat1}) = 1
Hazards of category 2 (N_{cat2}) = 4
Hazards of category 3 (N_{cat3}) = 3
Hazards of category 4 (N_{cat4}) = 1

CH_{sub} = 5.75

Fig. 1. General scheme for finding key information in the safety data sheet using the example of chloroform (Sigma-Aldrich).

Namieśnik for a detailed characterization of organic solvents commonly used in analytical laboratories [22].

CHEMS-1 parameterizes chemicals based on the following factors: oral toxicity towards rodents (HV_{ORAL}), inhalation toxicity towards rodents (HV_{INH}), carcinogenicity (HV_{CAR}), other hazardous effects (HV_{HE}), aquatic acute toxicity (HV_{FA}), aquatic chronic toxicity (HV_{FC}); and parameters related to exposure: biodegradability (HV_{BOD}), hydrolysis (HV_{HYD}), bioconcentration (HV_{BCF}) and volatility (HV_{VOL}) [22]. Hazard and exposure values range from 0 (lack of toxicity/exposure) to 5 (maximal toxicity/exposure). CHEMS-1 provides CH_{sub} value expressing all abovementioned properties of the assessed chemical, as the product of the sum of hazards related to toxicity by the sum of exposure factors (Eq.6). It means that the worst value is 600, while the best is 0 (nontoxic substance, like water):

$$CH_{sub} = \left(HV_{ORAL} + HV_{INH} + HV_{CAR} + HV_{HE} + HV_{FA} + HV_{FC}\right) \cdot \left(HV_{BOD} + HV_{HYD} + HV_{BCF} + HV_{VOL}\right) \tag{6}$$

According to CHEMS-1, the CH_{sub} value characterizing chloroform amounts to 103.8. Hence, the relative hazard characterizing the substance-of-interest is defined in the CHEMS-1 model as:

$$\frac{CH_{sub}}{CH_{CHCl3}} = \frac{CH_{sub}}{103.8} \tag{7}$$

Noteworthy, due to the different structure and framework, CH_{sub} values obtained with the WHN and CHEMS-1 models cannot be directly compared. Comparisons can be however made for the relative hazard values (CH_{sub}/CH_{CHCl3}), to analyze the overall consistency of models.

4. Evaluation of selected methods

The ChlorTox Scale was applied to assess and evaluate 14 analytical methods belonging to two groups that differ in specificity and research techniques used. The first group included seven methods of analyzing biological material for selected psychoactive compounds [23–28], the second group included seven methods of analyzing water samples for pesticides [29–35]. The assessment was conducted in parallel using two hazard models mentioned before, the simple WHN approach, and the more complex CHEMS-1 approach. Sigma-Aldrich was used as the preferred supplier of safety data sheets. To apply CHEMS-1 model, additional sources of data were applied [36]. The framework of the CHEMS-1 model was the same as in the previous work by Tobiszewski and Namieśnik [22]. The CHEMS-1 model was selected upon the assumption that it enables a more accurate and detailed description of hazards than a simple WHN model.

The methods within the groups differed significantly in the extraction techniques used. Input data on the amount of reagents were obtained directly from the text of articles, upon consultation with authors, or based on estimates supported by our laboratory experience. The detailed data on the assessment of individual reagents used by these methods are presented in Table 1 (biological samples) and Table 2 (environmental samples), while the overall comparison of individual methods in terms of the Total ChlorTox values, in relation to the WHN and CHEMS-1 hazard models, is presented in Fig. 2.

As shown in Fig. 2A, the overall comparison of methods for analyzing biological material in respect to the Total ChlorTox values is very similar for the simple WHN model and the more advanced CHEMS-1 approach. Both models indicate the LLE/HPLC and MAE/HPLC methods as definitively the worst. Their total risk has been estimated at about 5–6 g of chloroform per one sample measurement. The order of the other methods in the ranking is also the same for both models. The third worst method is MAE/UHPLC-MS, the fourth is DBS-MAE/UHPLC-MS, the fifth is MEPS/UHPLC-MS, the sixth is DI-SPME/UHPLC-MS, and the best of all is DBS-MAE/CE-MS. The reason for such a good assessment is the use of capillary electrophoresis as a separation technique instead of liquid chromatography, which is characterized by a negligible use of reagents [37,38].

In the case of environmental analysis methods (Fig. 2B), the overall picture is also similar for both hazard models, however, the ranking order is no longer identical. Both models indicate SS-LPME/GC–MS as the worst method, with the Total ChlorTox value close to 4 g, which is slightly less than in the case of the worst methods of biological material analysis. The WHN model shows SPE-DLLME/GC–MS as the second potentially dangerous method and RSE/GC–MS as the third, but the difference is very small. The CHEMS-1 model clearly indicates the RSE/GC–MS method as the second worst, and SPE-DLLME/GC–MS as the third worst. Further positions of the individual methods in the comparison are already consistent for both models. The best assessed method is DI-SPME/GC–MS, which is the only one characterized by a zero Total ChlorTox value, i.e. no chemical risk identified. The reason is the envi-

Table 1
Comparison of seven analytical methods for determination of selected psychoactive drugs in biological samples, in terms of the relative hazards in respect to chloroform (CH_{sub}/CH_{CHCl3}) obtained using the WHN and CHEMS-1 models, in terms of the mass of individual reagents used for one analysis (m_{sub}), and in terms of the ChlorTox values indicating the degree of predicted chemical risk.

Method	Stage	Compound	Relative hazard (WHN)	Relative hazard (CHEMS-1)	m_{sub} [mg]	ChlorTox (WHN) [g]	ChlorTox (CHEMS-1) [g]
LLE/HPLC [23]	LLE extraction	Sodium hydroxide	0.61	0.19	72.0	0.04	0.01
		n-Hexane	0.78	0.78	3 406.0	2.67	2.67
		Isoamyl alcohol	0.52	0.25	40.5	0.02	0.01
		Phosphoric acid	0.57	0.50	94.0	0.05	0.05
	HPLC Analysis	Acetonitrile	0.39	0.26	8 331.6	3.26	2.15
		Phosphoric acid	0.57	0.50	131.6	0.07	0.07
		Diethylamine	0.87	0.66	35.4	0.03	0.02
MAE/HPLC [23]	MAE extraction	Sodium hydroxide	0.61	0.19	72.0	0.04	0.01
		n-Hexane	0.78	0.78	4 054.5	3.17	3.18
		Isoamyl alcohol	0.52	0.25	48.6	0.03	0.01
		Phosphoric acid	0.57	0.50	94.0	0.05	0.05
	HPLC analysis	Acetonitrile	0.39	0.26	8 331.6	3.26	2.15
		Phosphoric acid	0.57	0.50	131.6	0.07	0.07
		Diethylamine	0.87	0.66	35.4	0.03	0.02
MEPS/UHPLC-MS [24]	MEPS extraction	Phosphoric acid	0.57	0.50	94.0	0.05	0.05
		Diethylamine	0.87	0.66	49.5	0.04	0.03
		Methanol	0.57	0.15	506.9	0.29	0.08
		Formic acid	0.57	0.41	427.0	0.24	0.18
		Ammonia water	0.91	0.80	17.6	0.02	0.01
		Ammonium formate	0.13	0.22	25.0	0.00	0.01
		Acetonitrile	0.39	0.26	19.7	0.01	0.01
	UHPLC-MS analysis	Acetonitrile	0.39	0.26	841.0	0.33	0.22
DBS-MAE/CE-MS [25]	DBS-MAE extraction	Acetic acid	0.43	0.02	105.0	0.05	0.00
		Sodium hydroxide	0.61	0.19	72.0	0.04	0.01
	CE-MS	Sodium hydroxide	0.61	0.19	0.0	0.00	0.00
		Methanol	0.57	0.15	6.1	0.00	0.00
		Acetic acid	0.43	0.02	1.1	0.00	0.00
		Isopropanol	0.35	0.07	18.7	0.01	0.00
		Formic acid	0.57	0.41	0.0	0.00	0.00
DBS-MAE/UHPLC-MS [26]	DBS-MAE extraction	Sodium tetraborate	0.30	0.12	15.0	0.00	0.00
		Hydrochloric acid	0.61	0.15	59.0	0.04	0.01
		Ethyl acetate	0.35	0.07	3 157.0	1.10	0.22
		Formic acid	0.57	0.41	12.2	0.01	0.01
	UHPLC-MS analysis	Formic acid	0.57	0.41	36.6	0.02	0.02
		Acetonitrile	0.39	0.26	1 572.0	0.62	0.41
MAE/UHPLC-MS [27]	MAE extraction	n-Hexane	0.78	0.78	2 593.8	2.03	2.03
		Isoamyl alcohol	0.52	0.25	32.4	0.02	0.01
		Formic acid	0.57	0.41	0.0	0.00	0.00
		Methanol	0.57	0.15	633.6	0.36	0.10
		Acetonitrile	0.39	0.26	39.3	0.02	0.01
	UHPLC-MS	Isopropanol	0.35	0.07	15.7	0.01	0.00
		Formic acid	0.57	0.41	0.0	0.00	0.00
		Acetonitrile	0.39	0.26	1 197.1	0.47	0.31
Di-SPME/UHPLC-MS [28]	DI-SPME	Acetonitrile	0.39	0.26	62.9	0.02	0.02
		Methanol	0.57	0.15	657.4	0.37	0.10
		Formic acid	0.57	0.41	0.0	0.00	0.00
	UHPLC-MS	Formic acid	0.57	0.41	0.0	0.00	0.00
		Acetonitrile	0.39	0.26	1 434.5	0.56	0.37

LLE/HPLC - liquid-liquid extraction with high performance liquid chromatography; MAE/HPLC - microwave assisted extraction with high performance liquid chromatography; MEPS/UHPLC - microextraction on packed sorbent with ultrahigh performance liquid chromatography; DBS-MAE/CE-MS - dry blood spot and microwave assisted extraction with capillary electrophoresis coupled with mass spectrometry; DBS-MAE/UHPLC-MS - dry blood spot and microwave assisted extraction with ultrahigh performance liquid chromatography coupled with mass spectrometry; MAE/UHPLC-MS - microwave assisted extraction with ultrahigh performance liquid chromatography coupled with mass spectrometry, DI-SPME/UHPLC-MS – direct immersion solid phase microextraction with ultrahigh performance liquid chromatography coupled with mass spectrometry; m_{sub} determines the mass of a reagent in a pure form needed to perform single measurement. It was assumed that the N number equals 100 in each case (see Eq.2). According to our laboratory experience, this is a reasonable number of measurements that can be carried out using the discussed extraction and separation techniques, without additional steps (preparation, calibration, rinsing), minimizing potential under- and overestimation of the actual risk. Water is not considered as a chemical reagent. The analyte standards are not considered in the assessment because their influence on the Total ChlorTox value would be negligible (much below 0.1 g).

Table 2

Comparison of seven analytical methods for determination of selected pesticides in environmental samples, in terms of the relative hazards in respect to chloroform (CH_{sub}/CH_{CHCl3}) obtained using the WHN and CHEMS-1 models, in terms of the mass of individual reagents used for one analysis (m_{sub}), and in terms of the ChlorTox values indicating the degree of predicted chemical risk.

Method	Stage	Compound	Relative hazard (WHN)	Relative hazard (CHEMS-1)	m_{sub} [mg]	ChlorTox (WHN) [g]	ChlorTox (CHEMS-1) [g]
DI-SPME/GC-MS [29]	DI-SPME extraction	Sodium chloride	0.00	0.00	2.7	0.00	0.00
	GC-MS analysis	Helium*	-	-	-	-	-
RSE/GC-MS [30]	RSE extraction	Ethyl acetate	0.35	0.07	902.0	0.31	0.06
		Chloroform	1.00	1.00	1 490.0	1.49	1.49
		Sodium chloride	0.00	0.00	9.3	0.00	0.00
	GC-MS analysis	Helium*	-	-		-	-
HS-SPME/GC-MS [31]	HS-SPME extraction	Acetonitrile	0.39	0.26	72.7	0.03	0.02
		DIL**	0.38	0.24	787.2	0.30	0.19
		Sodium chloride	0.00	0.00	0.2	0.00	0.00
	GC-MS analysis	Helium*	-	-	-	-	-
CSDF-ME/GC-MS [32]	CSDF-ME extraction	n-Hexane	0.78	0.78	85.2	0.07	0.07
		Methanol	0.57	0.15	63.4	0.04	0.01
		Sodium chloride	0.00	0.00	1.0	0.00	0.00
		Sodium hydroxide	0.61	0.19	1.0	0.00	0.00
	GC-MS analysis	Helium*	-	-	-	-	-
MSPE/GC-MS [33]	MSPE extraction	Sodium hydroxide	0.61	0.19	1.0	0.00	0.00
		Acetonitrile	0.39	0.26	628.8	0.25	0.16
	GC-MS analysis	Helium*	-	-	-	-	-
SS-LPME/GC-MS [34]	SS-LPME extraction	Sodium hydroxide	0.61	0.19	0.1	0.00	0.00
		Acetic Acid	0.43	0.02	0.2	0.00	0.00
		Acetonitrile	0.39	0.26	11 004.0	4.31	2.84
		Magnesium sulfate	0.00	0.04	6 000.0	0.00	0.25
		Sodium acetate	0.00	0.24	1 500.0	0.00	0.36
	GC-MS	Helium*	-	-	-	-	-
SPE-DLLME/GC-MS [35]	SPE-DLLME	Methanol	0.57	0.15	3 564.0	2.01	0.54
		Chlorobenzene	0.52	0.73	22.2	0.01	0.02
	GC-MS analysis	Helium*	-	-	-	-	-

DI-SPME/GC-MS - direct immersion solid phase microextraction with gas chromatography coupled with mass spectrometry; RSE/GC-MS - rotating sorptive extraction with gas chromatography coupled with mass spectrometry; HS-SPME/GC-MS - head space solid phase microextraction with gas chromatography coupled with mass spectrometry; CSDF-ME/GC-MS - continuous sample drop flow microextraction with gas chromatography coupled with mass spectrometry; MSPE/GC-MS - magnetic solid phase extraction with gas chromatography coupled with mass spectrometry; SS-LPME/GC-MS - switchable solvent-liquid phase microextraction with gas chromatography coupled with mass spectrometry; SPE-DLLME/GC-MS – solid phase extraction and dispersive liquid-liquid microextraction with gas chromatography coupled with mass spectrometry; m_{sub} determines the mass of a reagent in a pure form needed to perform single measurement. It was assumed that the N number equals 100 in each case (see Eq.2). According to our laboratory experience, this is a reasonable number of measurements that can be carried out using the discussed extraction and separation techniques, without additional steps (preparation, calibration, rinsing), minimizing potential under- and overestimation of the actual risk. Water is not considered as a chemical reagent. The analyte standards are not considered in the assessment because their influence on the Total ChlorTox value would be negligible (much below 0.1 g). (*) The hazard assessment for helium was not performed due to the difficulties arising from its different specificity and the marginal expected impact on the absolute value of Total ChlorTox (see the general guidelines how to apply ChlorTox Scale in the main text); (**) DIL -tetraethylene glycol-bis (3-benzylimidazolium) dibis(trifluoromethylsulfonyl)imide, the hazard for this reagent has been estimated in a simplified way due to the lack of data, relative hazard (CH_{sub}/CH_{CHCl3}) was estimated as the averaged values obtained for all other reagents classified in this table according to the WHN and CHEMS-1 models, respectively.

ronmentally friendly SPME extraction technique which in that case does not require the use of any organic solvents [39].

As can be seen from the analysis of Tables 1 and 2, the relative hazards posed by the individual chemicals under consideration (CH_{sub}/CH_{CHCl3} values) are in some cases quite similar for the WHN and CHEMS-1 models, but in some cases, differ quite significantly. The discrepancies recorded for methanol, acetonitrile and ethyl acetate are of the greatest importance for the results presented in Fig. 1. It is worth emphasizing that in the case of WHN, higher relative hazards were found than in the case of CHEMS-1. Therefore, the use of the WHN model in this case instead of CHEMS-1, assuming greater credibility of the CHEMS-1 model, entails the overestimation of risk. Nevertheless, this appears to be a generally better situation than the potential underestimation.

Despite significant differences in the structure of the models, the results obtained for WHN and CHEMS-1 should be considered qualitatively consistent, and some quantitative differences do not obscure the overall comparison of the considered methods. Regardless of the choice of the hazard model, the ChlorTox Scale indicated the same methods as the most risky, the same as moderately risky, and also the same as posing the least risk and thus most green.

5. Discussion

The approach we propose, although not perfect, has several important advantages. The chemical risk associated with the use of laboratory method gains a universal way of assessment and quantitative expression, the unit of which is the equivalent mass of chloroform. The results expressed in this way are easy to interpret and compare with each other, and the Total ChlorTox value describing the entire method can be used directly as one of the criteria for evaluating its greenness, whiteness, and in the formalized method validation protocols. The use of the ChlorTox Scale can deepen the evaluation of existing and newly developed methods, and make the discussion more objective.

An important advantage is also the fact that the ChlorTox Scale gives the freedom to choose preferred hazard estimation model, the choice of which can be adapted to specific circumstances. In addition, the ChlorTox Scale allows to use different ways of modeling the hazard for individual reagents used in the method, thus selecting the model most adequate to the specificity of a given substance. Due to the reference to the standard (chloroform), which is always considered with the same model as the substance under assessment, it is possible to maintain the consistency of the assessment process and the unit of scale. Chloroform,

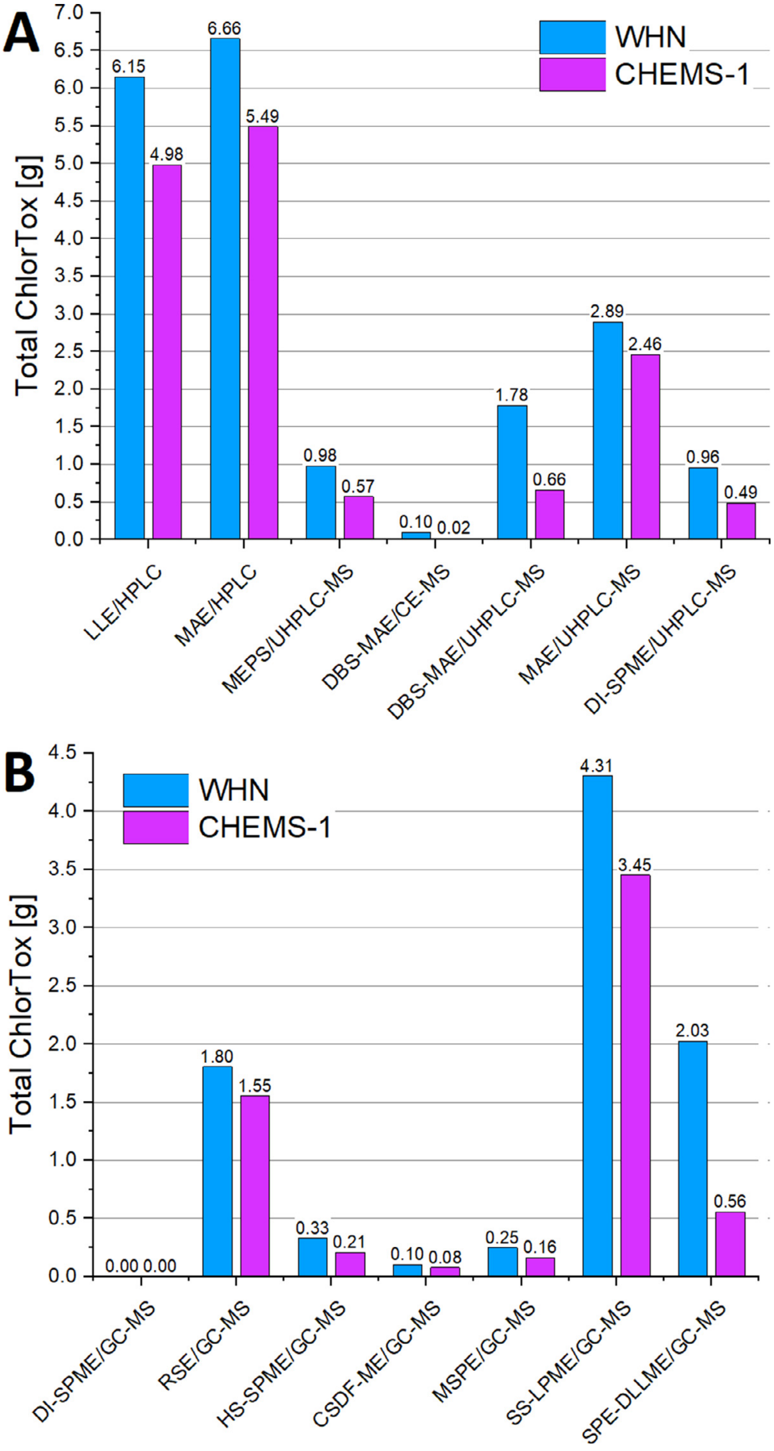


Fig. 2. The overall chemical risk quantified for the particular methods dedicated to biological samples analysis (A) and environmental samples analysis (B), using the Total ChlorTox values expressed in equivalent mass of chloroform per one analysis, obtained based on the WHN and CHEMS-1 hazard models.

due to the particularly complex structure of hazards, seems a good standard for implementing in various toxicity models.

The WHN model, proposed by us for simple estimating hazards and applying ChlorTox Scale, turned out to be substantively consistent with the more advanced CHEMS-1 model. Accessing key input data requires indeed very little effort, search engine on chemicalsafety.com website [20] or others can be used to find adequate safety data sheets. Choosing and indicating one preferred supplier of safety data sheet for the whole assessment (here Sigma-Aldrich) seems to be the simplest approach. However, another, more complex approach is possible – comparing all up-to-date sheets published for a given reagent by different entities, and then relying on the average WHN values. This approach can seem more reliable in terms of the assessment results, but requires the analysis of a larger set of data. In the near future we plan to collect and gather the safety data for most popular chemicals used in analytical laboratories in a dedicated ChlorTox Base. It will enable quick finding of average WHN values for the particular reagents and avoid the need for selecting the preferred data supplier and self-searching of data, which may be laborious.

Therefore, the use of the ChlorTox Scale together with the WHN model may be sufficient to assess risk and evaluate analytical methods in most cases. It is also possible to use more advanced hazard models, such as CHEMS-1, in situations when a more complex model structure is not a problem, or is desired due to the high importance of the assessment and its implications. A useful source of data for the use of more advanced hazard estimation models (CHEMS-1 and others) may be the Hazardous Substances Data Bank (HSDB) integrated with the PubChem database [36].

An inherent disadvantage of the ChlorTox Scale is that it is still a fairly general and estimative approach, and the calculated risk is purely theoretical. While we are able to count the amount of a given reagent accurately, we will never be able to reliably estimate and quantify the total hazard it poses. In practice, the potential risk determined by the ChlorTox Scale may be overestimated or underestimated. For instance, the approach described in Eq.1 does not take into account the risk associated with storage and contact with a dangerous chemical reagent used in a very small amount (negligible value of m_{sub}). In such a case appropriate quantities must be carefully weighed in order to prepare desirable dilution of a chemical, and this entail the exposure that can seem greater than reflected by the weighted mass of the substance. To account for this effect, Eq.1 would have to take a more complex form, including for example a constant that is independent of the amount of substance (m_{sub}) but dependent on its specificity. Unfortunately, this approach needs to be developed with further research and effort, and the resulting method would become more complicated to use. In our opinion, the currently presented approach is an optimal compromise between accuracy and simplicity. However, this effect should certainly be considered in the future when developing the ChlorTox Scale to be more accurate assessment tool. There are also other factors that are worth considering, but their analysis is not the purpose of this work. Notwithstanding, they will be carefully considered by us in the future to continuously develop and increase the credibility of the ChlorTox Scale.

Sometimes it can be technically difficult to calculate the exact quantity of reagents used, especially for the evaluation of a non-own method published in the literature. In this situation, the best solution is to contact the authors of the method with a request for the necessary information, and if this turns out to be impossible, to make possibly most reliable estimates. In the case of assessment based on such estimates, it is necessary to emphasize this fact when presenting the assessment results.

Finally, it should be emphasized that the ChlorTox Scale is aimed at evaluating methods based on two variables: the hazardous properties of the substance and its quantity. These parameters should be given in the description of each method. However, the ChlorTox Scale is not intended to assess processes or products that result from the use of a certain analytical method at a specific place and time. To assess the process, it is necessary to take into account an additional variable - the degree of prevention resulting from the applied protection measures, for example, the appropriate waste disposal procedure. The actual impact on the user's safety and environment depends on all three variables. In the case of a method that is only a certain recipe for performing an analysis, only the risk related to the parameters specified in the protocol can be estimated. Therefore, the ChlorTox Scale in its current form can be used to assess and compare methods published in the literature by estimating the risk of some adverse effects (defined as Chemical Risk), which can be minimized by providing appropriate protective means.

6. Conclusions

The quality of information provided by the ChlorTox Scale appears to be sufficient to assess the overall chemical risk and, on this basis, to evaluate, validate and compare analytical methods published in the literature. Indeed, its potential applicability is not limited to analytical laboratories. The ChlorTox Scale can be successfully employed anywhere where chemical reagents are used.

However, the results of the assessment should be treated semi-quantitatively, assuming a fair margin of uncertainty. Developing the formalized guideline for the interpretation of outcomes (ChlorTox values), adjusted to the method type and specificity, can help to judge which methods deserve to be called "green".

The application of the ChlorTox Scale should preferably be accompanied by the application of similar tools dedicated to assessing other types of risks that the method poses to the environment and to the user. They, however, yet need to be developed or improved. The currently used metric tools such as: Eco-Scale [8], AMGS [9], GAPI [10], ComplexGAPI [11], AGREE [12], AGREEprep [13], HEXAGON [14], RGB [15], RGB12 [16] and others, are based on quite subjective models and are of a less empirical nature than the ChlorTox Scale. Nonetheless, they can support the evaluation process and make the discussion more informative.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Paweł Mateusz Nowak: Conceptualization, Methodology, Formal analysis, Supervision, Writing – original draft, Writing – review & editing, Funding acquisition. **Renata Wietecha-Posłuszny:** Conceptualization, Investigation, Data curation, Methodology, Writing – review & editing. **Justyna Płotka-Wasylka:** Conceptualization, Investigation, Data curation, Methodology, Writing – review & editing. **Marek Tobiszewski:** Conceptualization, Investigation, Data curation, Methodology, Writing – review & editing.

Data availability

Raw data supporting the manuscript content is publicly available using the link: https://doi.org/10.57903/UJ/GIXTL9.

Acknowledgments

This research was funded by National Science Centre, Poland (P.M.N.; OPUS, 2020–2024, grant no. 2019/35/B/ST4/01022).

The authors would like to thank: Janusz Pawliszyn, Barbara Bojko, Fabiana Arduini, Maria Madej and Michał Woźniakiewicz for inspiration, scientific consultation, and support for the idea.

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